Our Heritage

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Nutritional and Antioxidant Capacities of Selected Plant Parts for Food Use

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Abstract

Antioxidants protect the body against oxidative stress by neutralizing free radicals. Plant contains rich number of polyphenols which are very potent natural's antioxidants. The study aimed at evaluating the nutritional and capacities of selected plant part for food use. Proximate, physio-chemical, phytol-chemical analysis of Hibiscus flower and leaves, were calculated. Hibiscus flower and leaves powder were incorporated to make extruded product. Since Hibiscus flower has significantly highest TPC 50.21±1.7 mg/100gm as Gallic acid equivalent and DPPH free radical scavenging activity 93.8±1.4 % values than Hibiscus leaves which has 39.23±1.57 mg100/gm as Gallic acid equivalent and DPPH free radical scavenging activity 88.76±1.43 %. Likewise, Hibiscus leaves has significantly highest TPC 39.23±1.57 mg/100gm Gallic as acid equivalent and DPPH free radical scavenging activity 93.8±1.4 % values. Therefore, Hibiscus flower and leaves were used to make extruded product. Therefore, extruded product with Hibiscus flower (T3 F (4%)) addition was accepted. And further Proximate, and Phyto- chemical analysis of extruded product with Hibiscus flower (T3 F (4%)) was calculated. So, the TPC 10.35 ± 1.25 mg/100gm as Gallic acid equivalent and DPPH free radical scavenging activity 53.99 ± 1.67% addition. Thus, food of plant origin serves as important source of minerals and vitamins in addition to protein and energy sources which are health conscious and consider as medicine.

Keywords: Hibiscus flower, Hibiscus leave, Phyto-chemical analysis, Proximate, Physiochemical, Antioxidant

ISSN: 0474-9030

Vol-68-Issue-1-January-2020

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Introduction

Antioxidants which are used nowadays are obtained mainly from two major routes; chemical synthesis and natural living source extraction. According to scientific research, severe toxicity caused by chemical synthetic antioxidants such as genotoxicity, carcinogenicity (Ito et al., 1986; Williams, Iatropoulos, & Whysner, 1999), or hepatotoxicity (Safer & Al-Nughamish, 1999) has been increasingly reported. Hence, the use of synthetic antioxidants is tending to decrease and needs replacement with other safer compounds. Meanwhile, natural antioxidants, derived mostly from their plants have been reported for high potential in prophylacsis and treatment of many degenerative diseases caused by chain oxidative reactions such as atherosclerosis, coronary heart disease, aging and cancer (Finkel & Holbrook, 2000). Additionally, plants can bean excellent source of natural antioxidants and can be effectively used in the food industry as a source of dietary supplements or as natural antioxidants to preserve the quality and improve the shelf-life of food products (Tiwari et al., 2009; Voon et al., 2012). Studies have demonstrated that the consumption of fruits, vegetables and seeds can be helpful to prevent the risk factors of many diseases due to the bioactive compounds.

Ascorbic acid is recognized for its important antioxidant effect (Anprung P 2011). ***Hibiscus rosa-sinensis*** **Linn (Malvaceae)** is a glabrous shrub widely cultivated in the tropics as an ornamental plant and has several forms with varying colors of flowers. In medicine, however the red flowered variety is preferred (Adhirajan et al., 2003). The leaves and flowers are observed to be promoters of hair growth and aid in healing of ulcers (Jadhav et al., 2009). Aerial part of *H. rosasinensis* has calcium channel blocking action (Gilani et al., 2005). Recent reports have also shown antiammonemic (Essa and Subramanian., 2007), antidiabetic (Venkatesh et al., 2008), hypolipidemic (Kumar et al., 2009), post-coital anti-fertility, cardio protective andwound healing activities

ISSN: 0474-9030

Vol-68-Issue-1-January-2020

(Gauthaman et al., 2006, Nayak et al., 2007). In the present study, the antioxidant property of 80% aqueous-ethanol crude extract of *H. rosasinensis* was examined by different in vitro analytical methodologies, such as total antioxidant activity determination by ferric thiocyanate, hydrogen peroxide scavenging, 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH) scavenging.

The present study entitled **"Nutritional and Antioxidants capacities of selected plant parts for food use"** was carried out with the following objectives

- Evaluation of physicochemical characteristics, nutritional composition and phytochemical analysis of selected plant parts.
- To find out the bioactive components (Ferulic, gallic, tannic, quecertine).

Materials and Methods:

Materials – Raw ingredients for analysis and product development were purchased from local market. The chemicals used for the analysis were of A.R.Grade and were purchased from Science Corporation.

Methods

Proximate Analysis

Estimation of Moisture:

Moisture was estimated by oven drying method. Weighed sample (approx 5gm) (W_2) pre-weighed petriplates(W_1) were kept in an oven for drying at 55 C for 5 hours. The samples were cooled in airtight dessicators to prevent moisture loss or gain from the environment. Drying was considered complete when readings of two consecutive weighing recorded at an interval of time did not vary by more than 5 mg (W_3). Moisture content was calculated by subtracting the dried weight from the sample weight and was expressed as percentage.

$$\% \text{ Moisture} = (W_2-W_3)/(W_2-W_1) \times 100$$

Estimation of Fat:

ISSN: 0474-9030

Vol-68-Issue-1-January-2020

Fat content in the sample was estimated by Soxhelt extraction method (**Ranganna,**2005). Moisture free sample was transferred to thimble which was then fixed into a stand and transferred to a pre-weighed Soxhelt beaker. The beaker was filled with petroleum ether. The beaker was then attached to Soxhelt apparatus and the sample was extracted for two hours at 60 C. At the same temperature the ether was evaporated for 2 hours after extraction. At the end of 4 hours the ether left was dried in hot oven at 100 C for 30 minutes.

The beaker was then cooled in dessicator and weighed. It gives the amount of ether soluble fat present in the sample.

Percent crude fat was calculated as under :

$$\% \text{ Crude fat} = (\text{weight of fat}) / (\text{weight of sample}) \times 100$$

Estimation of Crude Fibre:

Moisture and fat free sample (2 gm) was transferred to pre-weighed capsules tray and placed in the extraction vessel (carousel). The head of the carousel was attached to a condenser for reflux system (Ranganna,2005) . The sample was first washed with boiling dilute H_2SO_4 (1.25% v/v) to remove carbohydrate from sample from the sample. Washing is completed when the color of acid turns transparent. It was then washed with hot water. The sample was then washed with NAOH (1.25%w/v), till the washed solution exhibit no color. It was followed by hot water washing. Finally capsules' washing was done with petroleum ether (99%) to remove all organic compounds. It was followed by hot water washing.

The capsules were then dried for 1-2 hours in hot air oven at 100C cooled to room temperature and weighed. The capsules were then kept in pre-weighed crucibles for ashing in muffles furnace at 550C for 2 hours, till ashing is complete. Crucibles were cooled slowly in desiccators, to attain room temperature. The percent loss in weight was expressed as crude fibre.

$$\textbf{Percent Crude Fibre} = W_3(W_1 \times C) - (W_5 - W_4 - D) / (W_2) \times 100$$

ISSN: 0474-9030

Vol-68-Issue-1-January-2020

Where W_1- wt. of pre-dried empty capsules with lid.

W_2 - wt. of sample.

W_3 - wt. of capsule with residue after extraction.

W_4 – predried empty ashing crucible.

W_5 - total weight of crucible including ash.

C - wt.of blank capsule after extraction/wt. of blank capsule at start.

D- ash wt. obtained from the blank capsule itself.

Estimation of Protein:

The protein content of samples was determined by Kjeldahl procedure (described in Ranganna,2005). 0.5 gram of sample was digested with 5 gm of digestion mixture (10 parts potassium sulphate and 1 part copper sulphate) and 20 ml of conc. Sulphuric acid in Kjeldahl flask until the contents were carbon free. The digested sample was taken upto 100 ml. An aliquot of 10 ml was distilled with 20 ml of 30% sodium hydroxide and liberated ammonia was collected in 20 ml of boric acid containing 2-3 drops of mixed indicator (0.1% methyl red and 0.1% bromo-cresol green of 95% ethyl alcohol separately and mixed in the ratio of 1:5 respectively). The entrapped ammonia was titrated against titrated against 0.1N hydrochloric acid.

The Nitrogen content in the sample was calculated by the following expression:-

% Nitrogen =[(sample titre-blank titre)×normality of HCl×14×100×100] /(wt.of sample×aliquot taken for distillation×1000)

A conversion factor of 6.25 was used to convert nitrogen into protein content.

Estimation of Total Ash:

Ash determination was followed by the charing method (**Ranganna,**2005). 2 gm of sample taken in a silica crucible was ignited on a bunsen burner till the flumes stop coming (charing process) and then shifted to muffle furnace until clean ash was obtained

ISSN: 0474-9030

Vol-68-Issue-1-January-2020

. The temperature of furnace was raised to 550C. The weight of residue was noted and percent ash was calculated as under:

% Ash = (wt. of residue) / (wt. of sample) × 100

Estimation of Minerals:

For the determination of minerals mainly calcium, phosphorus and iron, acid soluble ash solution was used. (**Ranganna,**2005). Ash obtained from 2 gm of sample was boiled with 20 ml of 1:1 HCL for 30 mins and filtered through filter paper (whatman no. 42) washed with hot water until washings were acid free. The filtrate was made upto a known volume(100) and retained for the estimation of minerals.

(a) Calcium:- Calcium content in samples was determined by visual titration method (Ranganna,2005) . 20 ml of ash solution (obtained above) 25 ml of distilled water and 10 ml of saturated ammonium oxalate were taken in a beaker. To this,2 drops of methyl red indicator were added and the pH of the contents was adjusted to5.0 using dilute ammonia (1:1) and dilute acetic acid(1:4) solution . The contents were boiled and left at room temperature for overnight. Next day, the contents was through filter paper. The residue thus obtained was washed with hot distilled water until it became oxalate free. The filter paper was broken by a pointed glass rod and washed with 10 ml of hot dilute H_2SO_4 (1:4) followed by distilled water. The contents were heated to 80 C and and titrated against 0.01N $KMno_4$ to a stable pink colour . Finally, the filter paper was also dropped in the solution and titration was completed. Calcium was calculated as follows :-

Calcium (mg/100gm) = (Titre value × normality of $KMno_4$ × total volume of ash solution) / (ml of ash solution taken for estimation × wt. of sample taken for ashing)

(b) Phosphorus :- Phosphorus content was estimated according to the procedure described by (**Ranganna,2005).** 5 ml of molybdate reagent (25 gm of ammonium molybdate dissolved in 400 ml of distilled water) added to 500 ml of 10N H_2SO_4 and final volume made upto 500 ml with distilled water. This was allowed to stand for 10 minutes and the color was measured at 650 nmin a spectrophotometer. For standard,

ISSN: 0474-9030

Vol-68-Issue-1-January-2020

0.439 g potassium di-hydrogen phosphate and 10 ml of 10N H_2SO_4 were dissolved in water and volume was made to 1 liter. 5 ml of this solution was used for analysis.

The phosphorus content was calculated as follows:-

P (mg/100gm) = (mg of phosphorus in aliquot of ash solution taken for estimation × total vol.of ash solution) / (ml of ash solution taken for estimation × wt. of sample taking for ashing × 100)

Physico-chemical Analysis

Lab Value

Tristimulus color: Tristimulus color in terms of Hunter L, a, b values was measured using X-Rite spectrophotometer (USA) using D-65 illuminant and 10o observer. 'L' value represents lightness, 'a' value shows redness-greenness and 'b' value indicates blueness-yellowness of the samples.

Principle of operation: It measures spectral data—the amount of light energy reflected from an object at several intervals along the visible spectrum. These measurements result in a complex data set of reflectance values which are visually interpreted in the form of a spectral curve. Because a spectrophotometer gathers such complete color information, this information can be translated into colorimetric or densitometry data with just a few calculations. In short, a spectrophotometer is the most accurate, useful, and flexible instrument available.

A ***Lab*** **color space** is a color-opponent space with dimension ***L*** for lightness and ***a*** and ***b*** for the color-opponent dimensions, based on nonlinearly compressed CIE XYZ color space coordinates.

CIE *L*a*b** **(CIELAB)** is the most complete color space specified by the International Commission on Illumination (*Commission Internationale d'Eclairage*, hence its *CIE* initialism). It describes all the colors visible to the human eye and was created to serve as a device independent model to be used as a reference.

ISSN: 0474-9030

Vol-68-Issue-1-January-2020

The three coordinates of CIELAB represent the lightness of the color (***L**** = 0 yields black and ***L**** = 100 indicates diffuse white; specular white may be higher), its position between red/magenta and green (***a****, negative values indicate green while positive values indicate magenta) and its position between yellow and blue (***b****, negative values indicate blue and positive values indicate yellow)(McGuire,1992; Minolta,1998). The asterisk (*) after *L*, *a* and *b* are part of the full name, since they represent L*, a* and b*, to distinguish them from Hunter's L, a and b, described below.

Since the *L*a*b** model is a three-dimensional model, it can only be represented properly in a three-dimensional space.Two-dimensional depictions are chromaticity diagrams: sections of the color solid with a fixed lightness.

It is crucial to realize that the visual representations of the full gamut of colors in this model are never accurate; they are there just to help in understanding the concept. Because the red/green and yellow/blue opponent channels are computed as differences of lightness transformations of (putative) cone responses, CIELAB is a chromatic value color space.

Estimation of Phytochemicals / Antioxidant activity

Determination of Total Phenol Content

Total polyphenols were estimated as per procedure described by by Singleton *et. al.*(1999) using folin ciocalteu method , where 250 mg sample was taken in 10 ml of acetone and water (70:30 v/v) solution in a graduated test tube and heated on water bath at 70^{o}C for 10 min. The sample was brought to room temperature, centrifuged at 3000 rpm for 10 min. The supernatant (0.2 ml) was made up to 10 ml with distilled water. This solution was diluted 10 fold. Sample solution (5 ml) was mixed with saturated sodium carbonate (0.5 ml) and Folin-Ciocalteaue reagent (0.2 ml) and made up to 10 ml with distilled water. The absorbance was read at 765 nm after 60 min by UV visible double beam spectrophotometer (Model Evolution 600, Thermo Electron, US).

Determination of Radical Scavenging Activity

ISSN: 0474-9030

Vol-68-Issue-1-January-2020

The antioxidant activity of native and processed raw materials were also measured by the DPPH radical scavenging method (De Ancos, Sgroppo, Plaza, & Cano, 2002). An aliquot (0.10ml) of sample extract in methanol was mixed with 2 ml of methanolic 0.1 mM DPPH solution and the volume is made upto 5 ml with distilled water. The mixture was thoroughly vortex-mixed and kept in dark for 30 min. The absorbance was measured at 519 nm. The result was expressed as percentage of inhibition of the DPPH radical. The percentage of inhibition of the DPPH radical was calculated according to the following equation:

%inhibition of DPPH = [(Abs control-Abs sample)/(Abs control)] X 100

where, Abs control is the absorbance of the DPPH solution without the extracts.

Preparation of Extruded product from Hibiscus flower or leaves powder

Hibiscus flower or leaf powder (1.6%, 2.8% & 4%) was mixed in semolina after that water was mixed in the mixture of semolina and Feed the mixture into Extruder (Single screw extruder) Extruder rpm was 1544 rpm, Temp was 133 degree celcius, Cutter rpm was 12850 rpm .Cut into Pieces by extruder and Packed in PE films and Stored in dry place

Result and Discussion:

Nutritional and functional composition of fresh and dried hibiscus flower:

Particulars	Hibiscus Flower(fresh)	Hibiscus Flower(powder)
Moisture (%)	87.4±1.2	4.9±0.23
Protein (%)	6.7±0.59	21.2±1.87
Crude fat (%)	3.68±0.32	5.21±0.43
Crude fibre(%)	1.13±0.26	2.15±0.32
Ash (%)	0.43±0.04	1.89±0.1
Acidity (%)	0.86±0.032	1.06±0.2
L	33.27±1.74	12.1±1.23

ISSN: 0474-9030

Vol-68-Issue-1-January-2020

a	40.43±1.59	4.24±0.81
b	9.33±0.69	1.22±0.23
Calcium (mg/ 100 g)	25.1±2.3	169.21±2.2
Phosphorus (mg/ 100 g)	32±2.3	194±4.6
Iron (mg/ 100 g)	4.562±0.38	11.23±1.21
pH	6.9±0.2	6.22±0.25
DPPH(%)	89.6±1.34	93.8±1.4
TPC(mg/100 g gallic acid Eq.)	48.32±1.6	50.21±1.7
Vitamin C(mg/ 100 g)	13.43±1.07	36.13±2.1

Table No. 1 Nutritional and functional composition of fresh and dried hibiscus flower

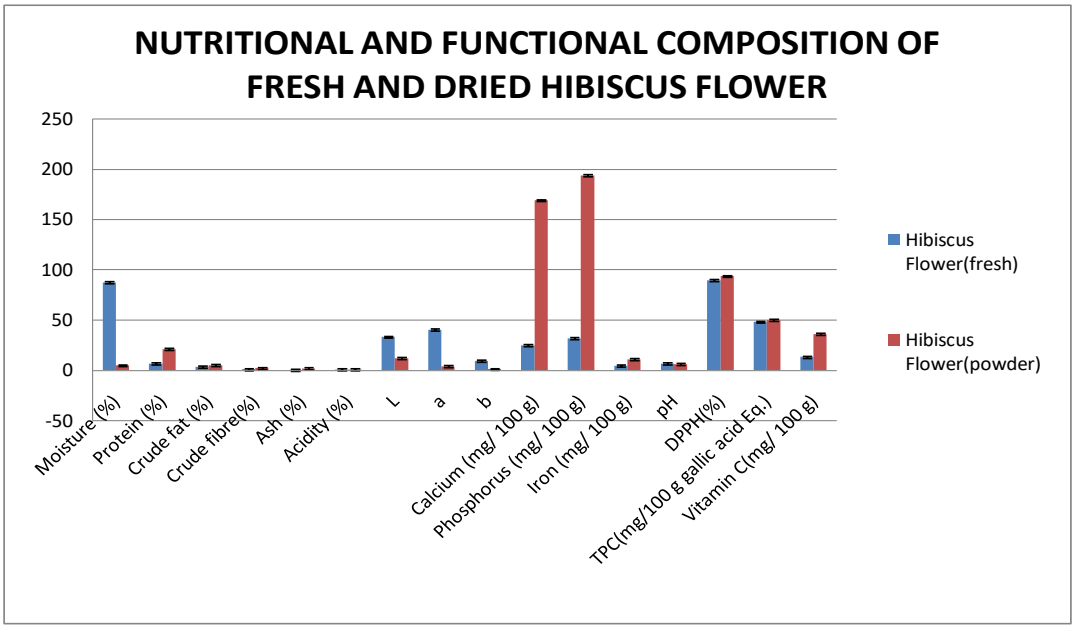

Fig: 1

Nutritional and functional composition of fresh and dried hibiscus leaves:

ISSN: 0474-9030

Vol-68-Issue-1-January-2020

Particulars	Hibiscus leaves(fresh)	Hibiscus leaves(powder)
Moisture (%)	79±1.1	3.31±0.12
Protein (%)	3.2±0.24	15.4±1.67
Crude fat (%)	1.32±0.11	3.51±0.22
Ash (%)	2.5±0.22	11.4±1.1
Crude fibre (%)	6.3±0.66	15.5±1.67
Acidity(%)	0.76±0.06	0.97±0.075
L	25.73±1.34	14.48±1.22
a	5.37±0.87	
b	4.77±0.75	2.58±0.2
Calcium (mg/ 100 g)	31±2.4	176.2±2.34
Phosphorus (mg/ 100 g)	22±1.7	166.2±4.2
Iron (mg/ 100 g)	10.47±1.39	62.35±2.78
pH	7.2±0.3	6.74±0.27
DPPH(%)	81.51±1.32	88.76±1.43
TPC(mg/100 g gallic acid Eq.)	32.61±1.4	39.23±1.57
Vitamin C(mg/ 100 g)	48±2.8	61.23±2.3

Table No. 2 Nutritional and functional composition of fresh and dried hibiscus leaves

ISSN: 0474-9030

Vol-68-Issue-1-January-2020

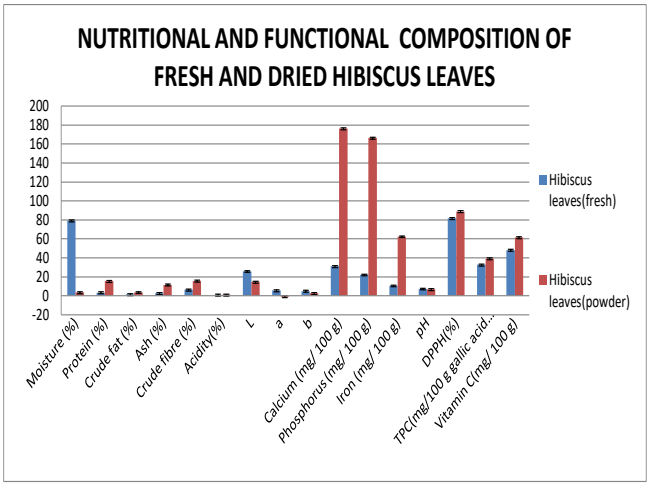

Fig: 2

Phytochemical/antioxidants activity of hibiscus flower and leaves:

Particulars	Hibiscus flower (powder)	Hibiscus leaves (powder)
Total Phenol Content (mg/gm gallic acid equivalent)	50.21±1.7	39.23±1.57
DPPH (%)	93.8±1.4	88.76±1.43
Vitamin C (mg/100g)	36.13±2.1	61.23±2.3

Table No 3 Phytochemical/antioxidants activity of hibiscus flower and leaves

ISSN: 0474-9030

Vol-68-Issue-1-January-2020

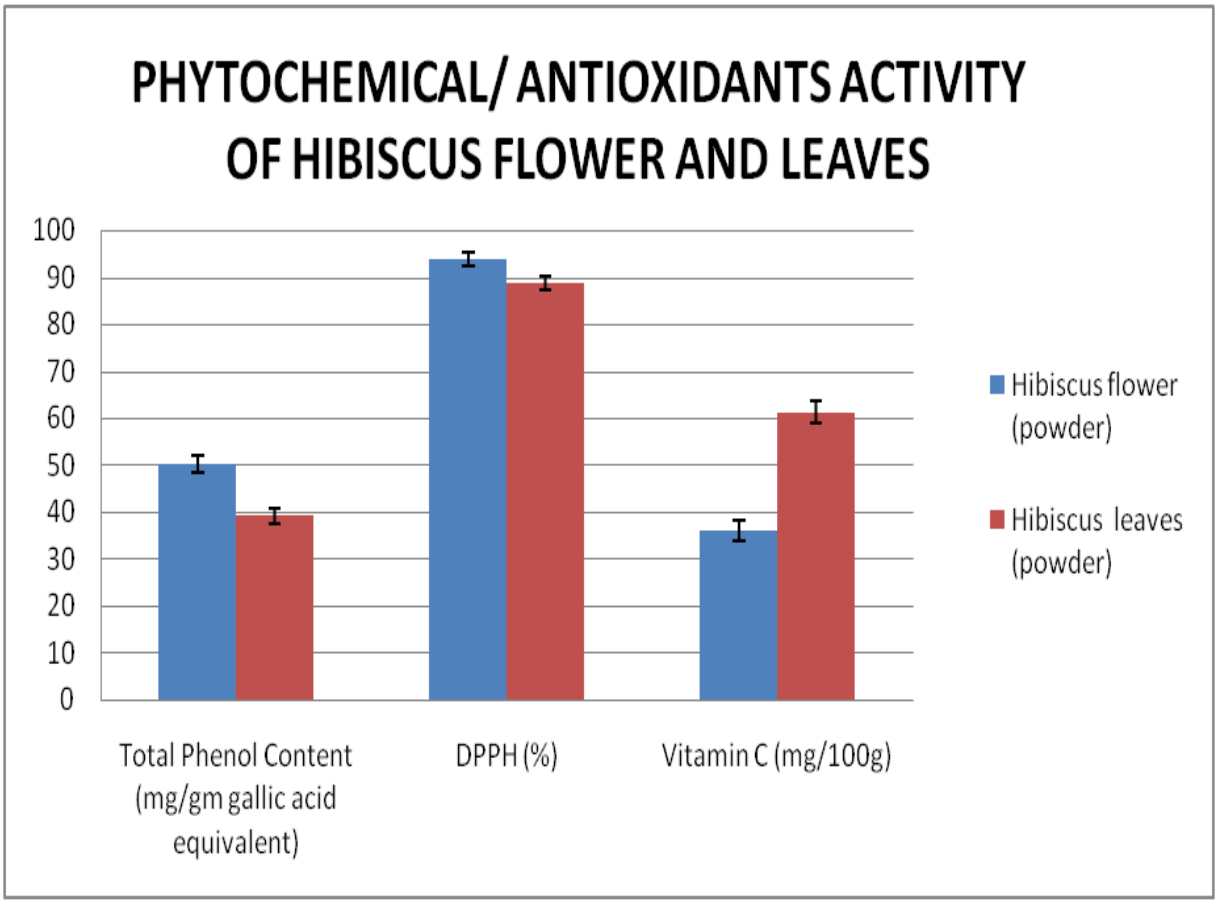

Fig: 3

Fig: 4 Extruded products with Hibiscus flower addition

ISSN: 0474-9030

Vol-68-Issue-1-January-2020

Fig: 5 Extruded products with Hibiscus leaves addition

Effect of Hibiscus flower addition on sensory quality of Extruded product:

Sample	Colour	Flavour	Texture	Overall Acceptability
Control	8.5	8.5	8.5	8.5
T1F (1.6%)	7.5	7	7.5	7.5
T2F (2.8%)	7.5	7	8	8
T3 F (4%)	8.5	8	8.5	8.5

Table No. 4 Effect of Hibiscus flower addition on sensory quality of Extruded product

ISSN: 0474-9030

Vol-68-Issue-1-January-2020

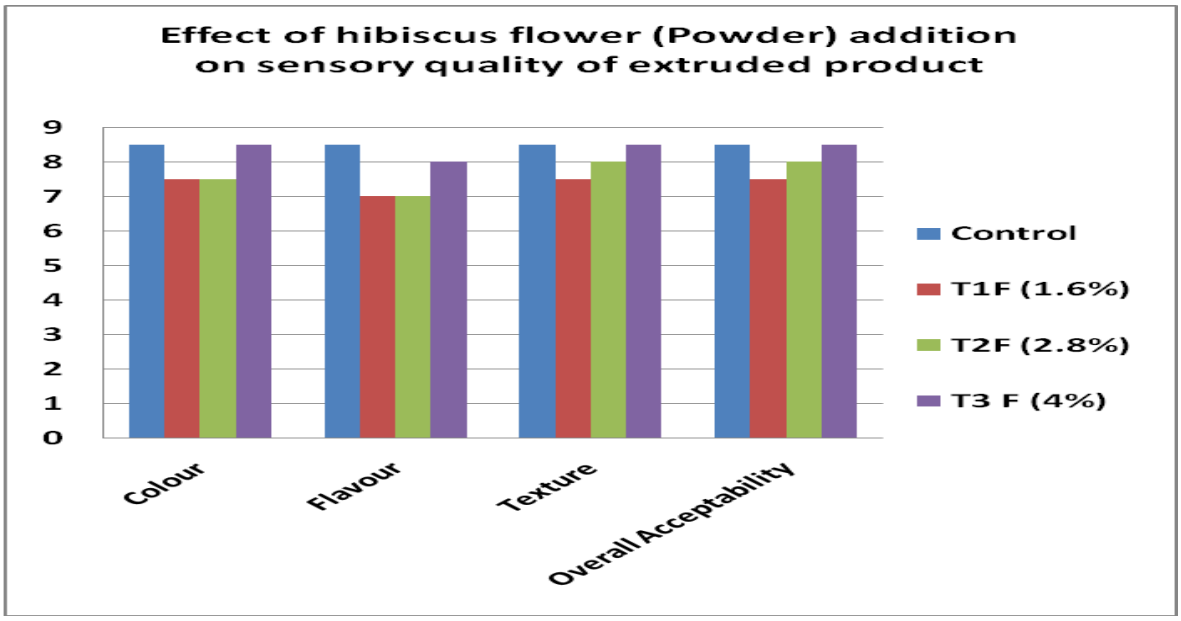

Fig: 6

Effect of Hibiscus leaves addition on sensory quality of Extruded product:

Sample	Colour	Flavour	Texture	Overall Acceptability
Control	8.5	8.5	8.5	8.5
T1L (1.6%)	8	8	8	8
T2L (2.8%)	8.25	7	8.25	7
T3L (4%)	8.5	6	8.5	6.5

Table No. 5 Effect of Hibiscus leaves addition on sensory quality of Extruded product

ISSN: 0474-9030

Vol-68-Issue-1-January-2020

Nutritional, functional and antioxidants / phytochemical composition in control and developed extruded product

Particulars	Control	Developed product (T3F 4%)
Moisture (%)		
Protein (%)		
Crude fat (%)		
Ash (%)		
Crude fibre (%)		
L	33.25 ±0.73	29.4 ±1.35
A	3.78 ±0.05	6.56 ±0.28
B	4.52 ±0.07	4.74± 0.25
Total Phenol Content (mg/gm gallic acid equivalent)	7.67 ± 0.29	10.35 ± 1.25
DPPH (%)	49.47 ± 1.23	53.99 ± 1.67

Conclusion:

The Proximate, physico-chemical,phyto- chemical analysis of Hibiscus flower and leaves, were evaluated . The antioxidant activity of various medicinal plants can be determined precisely, conveniently, and quickly Using total phenolic contents, DPPH free radical scavenging activity and vitamin C by 2,6 -dichlorophenol-indophenol dye titration methods . This study has shown the proximate, mineral, vitamin, phytochemical compositions of Musa paradisiacal bract(Banana flower) as a rich source of minerals coupled with the presence of phytochemicals and nutrients.

The Results of phytochemical analysis of the flower extract of *Hibiscus rosa sinensis*, flavonoids , phenolic compounds are found in the extract. Since phenolic compounds and

ISSN: 0474-9030

Vol-68-Issue-1-January-2020

flavonoids are responsible for the antioxidant activity, the amounts present in the extract are high indicating good antioxidant activity. The presence of phenolic compounds in the flower contributed to its antioxidant activity and thus usefulness of the *Hibiscus rosa sinensis* plant as a medicine it can play a therapeutic role against number of epidemic and pathogen born diseases.

Since Hibiscus flower and leaves have significantly highest TPC and DPPH free radical scavenging activity. Hibiscus flower with TPC 50.21±1.7 mg/100gm as gallic acid equivalent and DPPH free radical scavenging activity 93.8±1.4 % values and Hibiscus leaves with TPC 39.23±1.57 mg100/gm as gallic acid equivalent and DPPH free radical scavenging activity 88.76±1.43 was found to be significantly higher than Guava leaves and Banana flower . Therefore, Hibiscus flower and leaves powder were incorporated to make extruded product. The over all acceptibility of sensory evaluation of extruded product with Hibiscus leaves addition was significantly less than Hibiscus flower .Therefore extruded product with Hibiscus flower (T3 F(4%)) addition was accepted On the basis of sensory evaluation further Proximate, and phyto- chemical analysis of extruded product with Hibiscus flower (T3 F(4%)) was calculated .So the TPC 10.35 ± 1.25 mg/100gm as gallic acid equivalent and DPPH free radical scavenging activity 53.99 ± 1.67% values was measured in extruded product with Hibiscus flower (T3 F(4%)) addition when compared with the control extruded product The extruded product with Hibiscus flower T3 F(4%) addition was found rich in bioactive components in terms of DPPH, TPC when compared with the control extruded product

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ISSN: 0474-9030

Vol-68-Issue-1-January-2020

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ISSN: 0474-9030

Vol-68-Issue-1-January-2020

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